

Abundance and variation of colloidal organic phosphorus in riverine, estuarine, and coastal waters in the northern Gulf of Mexico

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Abstract

The abundance of colloidal organic phosphorus (COP) and colloidal inorganic phosphorus (CIP) was quantitatively determined using an ultrafiltration permeation model for riverine, estuarine, and coastal waters in the northern Gulf of Mexico. Dissolved inorganic phosphorus (DIP) was the dominant species in Mississippi and Pearl River waters, while dissolved organic phosphorus was dominant in marine environments. The abundance of COP was highest in the Pearl River (up to 88%), followed by the Mississippi Bight (~67%), and the Bay of St. Louis (~59%), but was lowest in the Mississippi River (41–50%). These variations highlight the roles of terrestrial inputs, autochthonous production, and anthropogenic activities in controlling the COP abundance in different aquatic environments. In the Gulf of Mexico, COP abundance generally decreased with increasing depth and coincided with chlorophyll *a*, reflecting the production of COP by phytoplankton and remineralization during downward transportation in the water column. The colloidal organic C:P molar ratios were substantially higher than the Redfield ratio but consistently lower than those of the bulk dissolved organic matter except for the Mississippi River, indicating a diagenetically fresher or younger COP pool. The percentage of CIP in the DIP pool was generally negligible or very low ($\leq 3\%$) except in the Pearl River, where CIP abundance was as high as 27–47%, likely contributed from colloidal soils, minerals, and iron oxyhydroxides. We hypothesize that high COP abundance and seasonal P-limitation play an important role in regulating the biogeochemical cycling of P and the development of hypoxia in the northern Gulf of Mexico.

Phosphorus (P) is an essential macronutrient that plays a key role in regulating primary production in aquatic environments (Benitez-Nelson 2000). Among P species, phosphate is the form most readily utilized by aquatic organisms and can be depleted before inorganic nitrogen (N) species and, thus, may limit primary production in various aquatic ecosystems (Cotner et al. 1997; Murrell et al. 2002; Sylvan et al. 2006). To reduce nutrient stress and P limitation, microorganisms and phytoplankton may utilize dissolved organic phosphorus (DOP) through hydrolyzation by various enzymes, such as alkaline phosphatase and 5'-nucleotidase, under conditions of low phosphate concentration (Karl and Craven 1980; Ammerman and Azam 1985; Björkman and Karl 1994). Within the DOP pool, colloidal organic phosphorus (COP) is thought to be a substantial and active component affecting DOP bioavailability and cycling pathways (Kolowith et al. 2001; Karl and Björkman 2002). The chemical and phase speciation of P and its interchange between inorganic and organic and between dissolved, colloidal, and particulate phases are likely to play an important role in regulating nutrient limitation and water quality in aquatic environments. However, phase partitioning of P and its utilization pathways and biogeochemical cycling remain poorly understood, and the abundance of COP and its distribution and variation in natural waters have rarely been systematically studied.

The cross-flow ultrafiltration technique has been widely used to determine the abundance and size distribution of organic matter and trace elements and to isolate colloidal materials for chemical, isotopic, and molecular characterization (Benner 2002; Guo and Santschi 2007). Although

quantitative determination of colloidal abundance of chemical species using ultrafiltration methods has been controversial, the consensus is that the conventional method based on discrete sampling overestimates colloidal abundance and is susceptible to operating conditions, such as solute recoveries and concentration factors (Guo and Santschi 2007), and that time-series sampling coupled with the permeation model is required for quantitative determination of colloidal abundance in natural waters (Guo and Santschi 1996; Guo et al. 2000; Belzile and Guo 2006). Nevertheless, colloidal abundance data derived from permeate time-series sampling coupled with the permeation model are still scarce and are mainly reported for colloidal organic carbon (COC; Belzile and Guo 2006; Cai et al. 2008a). The natural abundance and distributions of COP in aquatic environments are poorly quantified, and the available data are mostly derived from the conventional method based on concentration differences among permeate, retentate, and initial samples. The existing data are also highly variable, with results ranging from undetectable to nearly 100% COP as a result of complicating factors such as low DOP concentration, poor ultrafiltration membrane calibration and performance, and, most importantly, defects of conventional colloidal calculation methods (Ridal and Moore 1990; Bauer et al. 1996; Rinker and Powell 2006).

The northern Gulf of Mexico is experiencing profound influences of nutrients and organic loadings from river runoff and coastal erosion; these events are causing hypoxia, eutrophication, and other potential environmental problems (Rabalais et al. 2002; Brunner et al. 2006). Although the nitrate export from the Mississippi River has declined significantly since 1990, the annual total P flux has remained stable or has increased slightly (Goolsby et al. 2000; Turner et

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al. 2007). Recent studies have shown that, in addition to N, P may also play an important role in hypoxia development in this region (Sylvan et al. 2006, 2007). Unfortunately, studies on the biogeochemistry of DOP and COP in the northern Gulf of Mexico remain scarce.

To better understand the biogeochemical cycling of P and its role in hypoxia formation in aquatic ecosystems, especially in the northern Gulf of Mexico, quantitative determinations of chemical speciation and phase partitioning of P are essential. In this study, we have applied the ultrafiltration technique combined with the ultrafiltration permeation model to quantify the abundance of both colloidal inorganic phosphorus (CIP) and COP and to determine their vertical and spatial distributions in the study areas. Samples were collected from a number of different aquatic environments in the northern Gulf of Mexico, including the Mississippi, Atchafalaya, and Pearl Rivers; the Bay of St. Louis; the Mississippi River plume; the Mississippi Bight; and the Gulf of Mexico.

Methods

Sampling sites—The Mississippi River is the largest river system on the North American continent and drains ~40% of the continental United States and part of Canada (Milliman 1991). The Mississippi River is the primary source of freshwater, nutrients, and organic matter for the northern Gulf of Mexico (Goolsby et al. 2000; Dagg et al. 2004), of which 30% of freshwater is discharged through the Atchafalaya River (Turner et al. 2007). The Mississippi River is heavily influenced by human activities, such as intensive farming, dams on the primary tributaries, and flood control levees along the main river channel (Keown et al. 1986).

The Mississippi River plume is a eutrophic and highly productive region owing to high nutrient influx from the Mississippi River (Lohrenz et al. 1997). The eutrophication and high primary production in the plume, along with water column stratification, have caused widespread hypoxia on the Louisiana–Texas continental shelf (Rabalais et al. 2002). The Mississippi River plume experiences a seasonal shift of nutrient regulation, from P limitation during spring and early summer to N limitation in the fall (Sylvan et al. 2006, 2007).

The Pearl River originates in the east–central portion of the state of Mississippi and discharges into the Mississippi Bight. It is a small blackwater, forested river and has a drainage basin of 22,690 km². River swamp and salt marsh are widespread along the riparian zone and river delta (U.S. Geological Survey, http://ms.water.usgs.gov/ms_proj/eric/pearl.html). Compared to the Mississippi River, the Pearl River drainage basin is far less populated, with less human influence.

The Bay of St. Louis is a shallow, semi-enclosed estuarine system connected to the Mississippi Sound through a narrow passage. It receives freshwater and nutrient inputs from two small blackwater rivers, the Jourdan and Wolf Rivers, and several bayous. In addition to the riverine nutrient sources, the Bay of St. Louis also receives nutrients from anthropogenic point sources, including the DuPont DeLisle titanium dioxide plant, two

sewage treatment outfalls, and a casino, as well as non-point sources, such as leaking septic tanks and recreational and agricultural activities within the Jourdan and Wolf watersheds (Phelps 1999).

The Mississippi Bight is a shallow coastal system separated from the Mississippi Sound and Chandeleur Sound by a series of barrier islands, with freshwater discharge from a number of coastal rivers and bayous along the Louisiana, Mississippi, and Alabama coasts. Among those rivers, the Pearl River and the Pascagoula River deliver most of the freshwater. Low-oxygen events and hypoxia have been reported for the Mississippi Bight (Brunner et al. 2006).

The Gulf of Mexico is a semi-enclosed oceanic system hydrographically characterized by the presence of anticyclonic warm core rings that spin off the Loop Current and cyclonic cold core rings often found near the perimeter of these warm core rings. Warm core rings are depleted in nutrients in surface water and generally penetrate to a depth of more than 100 m, while cold core rings have much shallower nutriclines and higher primary production (Biggs 1992).

Sample collection—Water samples were collected from all sites described above (Table 1; Fig. 1). Surface-water samples (~0.5 m below water surface) from rivers were collected through in situ pumping and filtered through a pre-rinsed 0.45- μ m polycarbonate cartridge (Osmonics). About 40 liters of filtrate were collected for ultrafiltration; aliquots of filtrate and total waters were also collected for the measurement of nutrients, dissolved organic carbon (DOC), and other parameters (Cai et al. 2008b). Vertical profile samples were collected from the Gulf of Mexico and Mississippi River plume using Niskin bottles mounted on a conductivity–temperature–depth (CTD) rosette system aboard the R/V *Seward Johnson*.

The Mississippi River water samples were collected near the U.S. Geological Survey hydrological station at Baton Rouge, Louisiana, and the Atchafalaya River was sampled upstream of the Interstate 10 bridge (Fig. 1). The Pearl River was sampled at Bogalusa, Louisiana, and Stennis Space Center, Mississippi. The Bay of St. Louis water sample was collected at a station near the mouth of the bay. The Mississippi Bight water sample was collected near the University of Southern Mississippi buoy Sta. USM3M01 (30.04°N, 88.65°N). Water samples from the Mississippi River plume (Sta. 8B and Sta. 12A) and the Gulf of Mexico were collected aboard the R/V *Seward Johnson* during May 2006. The Gulf of Mexico sampling sites at Sta. S1 and Sta. S3 represent the cold core ring, and Sta. S2 is in a warm core ring (Table 1).

Ultrafiltration—An ultrafiltration system equipped with a spiral-wound, 1-kDa cartridge (Amicon S10Y1) and Teflon pump head and tubing was used. Details of the cartridge calibration, cleaning, and ultrafiltration procedure have been described elsewhere (Guo and Santschi 1996; Guo et al. 2000). Briefly, the ultrafiltration cartridge was calibrated with 1.3 kDa vitamin B₁₂ and 4.4 kDa fluorescein isothiocyanate–tagged dextran to check the

Table 1. Sampling locations and the concentration factor (*CF*) used for ultrafiltration.*

Sample ID	Latitude (°N)	Longitude (°W)	Sampling date	Salinity	<i>CF</i>	DOC (μmol L ⁻¹)	DOP (μmol L ⁻¹)	DIP (μmol L ⁻¹)
Mississippi River-1	30.4380	91.1922	25 Jan 07	0	18	291	0.19	1.71
Mississippi River-2	30.4380	91.1922	27 Aug 07	0.2	22	286	0.24	2.90
Atchafalaya River	30.3931	91.6796	25 Oct 07	0.3	17	309	0.13	3.50
Pearl River-1 (Bogalusa)	30.7917	89.8219	18 Dec 07	0.1	31	257	0.16	0.70
Pearl River-2 (Stennis)	30.3487	89.6413	12 Jan 07	0	23	790	0.25	0.67
Pearl River-3 (Stennis)	30.3487	89.6413	12 Jul 07	0	23	1056	0.31	0.88
Bay of St. Louis	30.3018	89.3270	11 Sep 07	21.6	29	330	0.35	1.47
Mississippi Bight	30.0387	88.6531	14 Dec 07	34.9	17	100	0.17	0.046
Gulf of Mexico								
S1-2 m	25.8737	92.5150	01 May 06	36.4	21	75	0.062	0.011
S1-75 m	25.8737	92.5150	02 May 06	36.5	20	78	0.068	0.030
S1-140 m	25.8737	92.5150	02 May 06	35.9	26	60	0.053	0.89
S2-2 m	26.9095	89.9907	03 May 06	34.8	25	84	0.077	ND
S2-65 m	26.9095	89.9907	03 May 06	36.2	22	83	0.078	ND
S2-140 m	26.9095	89.9907	04 May 06	36.6	32	77	0.076	ND
S3-2 m	27.8698	38.6408	05 May 06	36.6	29	84	0.058	ND
S3-90 m	27.8698	38.6408	06 May 06	36.4	28	86	0.065	0.030
S3-140 m	27.8698	38.6408	06 May 06	36.4	30	74	0.066	0.46
S3-500 m	27.8698	38.6408	07 May 06	35.0	24	64	0.055	1.57
Mississippi River Plume								
12A-2 m	28.8317	89.7492	08 May 06	ND	24	183	0.081	0.020
8B-2 m	28.8862	90.6660	08 May 06	29.9	24	163	0.067	0.011

* DOC, dissolved organic carbon; DOP, dissolved organic phosphorus; DIP, dissolved inorganic phosphorus; ND, not detected.

membrane integrity. The S10Y1 membrane had an average rejection rate of 91% for the 1.3-kDa vitamin B₁₂ and of 99% for the 4.4-kDa dextran, resulting in an apparent molecular weight cutoff of ~1 kDa based on a 90% rejection rate. Before sample processing, the ultrafiltration cartridge was consecutively cleaned with 1% Micro detergent, 0.05 mol L⁻¹ NaOH, and large volumes of Milli-Q water and preconditioned with a 2-liter water sample (Guo and Santschi 1996). About 20 liters of filtrate sample was ultrafiltered at a back pressure of ~276 kPa. Discrete time-series permeate samples were collected at different concentration factors for use in applying the permeation model to quantify the abundance of low-molecular-weight (LMW, <1 kDa) and colloidal fractions. Two consecutive washes with Milli-Q water were performed after ultrafiltration. Initial sample, integrated permeate, cartridge wash, and retentate samples were collected before and/or after ultrafiltration, allowing the determination of a P mass balance and apparent colloidal fraction for comparison with the true colloidal fraction derived from the ultrafiltration permeation model. Satisfactory P mass balance results (98% ± 2%) obtained for all samples exclude the possibility of system contamination, except for one sample that had a slightly lower recovery (93%), resulting largely from loss of COP to the ultrafiltration system under a concentration factor of >22.

Determination of P—The autoclave-assisted acid persulfate method was used for the oxidation of DOP (Koroleff 1983; Ridal and Moore 1990). One milliliter of acidified 50 g L⁻¹ K₂S₂O₈ solution (pH = 1) was mixed with a 9-mL aliquot sample in a Teflon vial with a Teflon screw cap, and the

resulting solution was autoclaved at 125°C for 90 min. Total dissolved phosphorus (TDP) concentrations were measured by the standard phosphomolybdenum blue method with 5-cm quartz cuvettes on a Gary 300 ultraviolet-visible dual-beam spectrophotometer following the reduction of arsenate, while phosphate (DIP) concentrations were measured without acid persulfate oxidation (Cai et al. 2008b). The detection limits were generally 10 nmol L⁻¹, with a precision of better than 2% for both DIP and TDP. Concentrations of DOP were calculated from the difference between TDP and DIP. Concentrations of DOC were measured by a high-temperature combustion method on a Shimadzu TOC-V analyzer (Guo et al. 1995). Precision was better than 2% and accuracy was within 1%, based on DOC working standards.

Calculation of colloidal fraction using concentration difference and ultrafiltration permeation model—According to the conventional method (Bauer et al. 1996), apparent COP concentration, *COP_C*, can be calculated as follows:

$$[COP_C] = \left([DOP]_{Retentate} - [DOP]_{Integrate} \right) / CF \quad (1)$$

where $[DOP]_{Retentate}$ and $[DOP]_{Integrate}$ are the DOP concentrations in the retentate and integrated permeate solutions, respectively, and *CF* is concentration factor. This conventional calculation is highly dependent on the *CF* used, since DOP in the integrated permeate increases with increasing *CF*, resulting in an overestimation of natural colloidal abundance (Guo and Santschi 1996).

The ultrafiltration permeation model has been used to describe the permeable solute behavior during the ultrafiltration (Guo and Santschi 1996). At any point during the

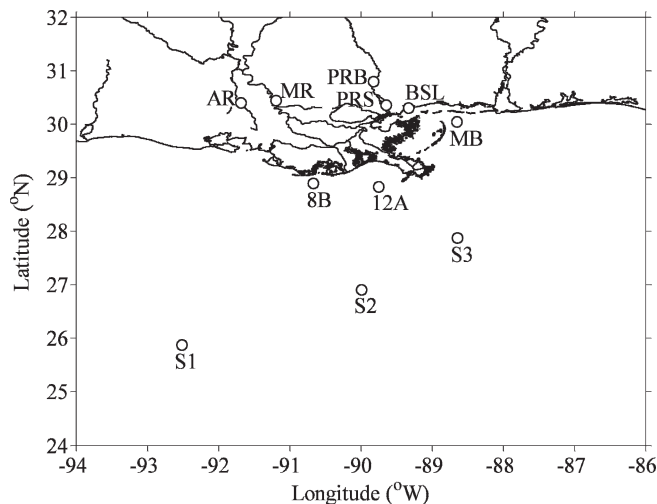


Fig. 1. Sampling locations in the Mississippi and Pearl Rivers, Bay of St. Louis, Mississippi Bight, and the Gulf of Mexico. AR = Atchafalaya River; MR = Mississippi River; PRB = Pearl River at Bogalusa, Louisiana; PRS = Pearl River at Stennis Space Center, Mississippi; BSL = Bay of St. Louis; MB = Mississippi Bight; Sta. S1, S2, and S3 are located in the Gulf of Mexico, while Sta. 8B and 12A are located in the Mississippi River Plume.

ultrafiltration, the relationship between permeable DOP or DIP concentration in the permeate, C_p , and the CF can be described as

$$\ln(C_p) = (1 - P_c)\ln(CF) + \ln(C_L^0) \times P_c \quad (2)$$

where P_c is the permeation coefficient for LMW, or permeable, DOP or DIP and where C_L^0 is the LMW DOP or DIP concentration in the initial sample. A linear relationship between $\log(C_p)$ and $\log(CF)$ indicates a constant permeation (constant P_c) during the entire ultrafiltration for the LMW DOP and DIP (Guo and Santschi 1996; Guo et al. 2000). As a result of the constant permeation, values of P_c and C_L^0 can be calculated from the values of the slope and intercept of the linear relationship between $\log(C_p)$ and $\log(CF)$, thus:

$$P_c = 1 - \text{slope} \quad (3)$$

Table 2. Colloidal phosphorus (P) concentrations and percentages derived from the ultrafiltration permeation model (COP_M and CIP_M) and the conventional method by concentration difference (COP_C).*

Sample ID	Recovery (%)	CIP_M ($\mu\text{mol L}^{-1}$)	% CIP_M	COP_M ($\mu\text{mol L}^{-1}$)	% COP_M	COP_C ($\mu\text{mol L}^{-1}$)	% COP_C	Difference (%)
Mississippi River-1	97	0.067	3.9	0.095	50	0.10	55	10
Mississippi River-2	93	0.090	3.1	0.12	50	0.11	47	-6
Atchafalaya River	98	0.116	3.3	0.053	41	0.068	52	27
Pearl River-1	98	0.329	47	0.14	87	0.14	90	3
Pearl River-2	100	0.181	27	0.22	89	0.24	95	7
Pearl River-3	101	0.308	35	0.27	87	0.30	97	11
Bay of St. Louis	98	0.037	2.5	0.21	59	0.21	61	3
Mississippi Bight	96	0.0006	1.2	0.11	67	0.12	72	7
Average \pm SD	98 \pm 2	141 \pm 122	15 \pm 18	0.15 \pm 0.07	66 \pm 19	0.16 \pm 0.08	71 \pm 20	8 \pm 9

* CIP, colloidal inorganic phosphorus; COP, colloidal organic phosphorus; SD, standard deviation.

and

$$C_L^0 = \exp(\text{intercept})/P_c \quad (4)$$

The model-derived concentration of COP (COP_M) or colloidal inorganic phosphorus (CIP_M), which is independent of CF , can then be calculated from the difference between C_L^0 and the initial bulk DOP or DIP concentration (Guo and Santschi 1996).

Results

Concentrations of DIP and DOP—DIP concentrations in the water samples (Table 1) were closely related to the environmental setting of each location. The highest DIP concentrations (1.7–3.5 $\mu\text{mol L}^{-1}$) were measured in the Mississippi River, which experiences heavy human influence, while the Pearl River and the Bay of St. Louis had medium DIP concentrations (0.7–1.5 $\mu\text{mol L}^{-1}$), reflecting less human influence. Surface-water samples from marine environments, including the Mississippi Bight, the Mississippi River plume, and the open Gulf of Mexico, had very low DIP concentrations ($<0.05 \mu\text{mol L}^{-1}$). Low DIP concentrations in coastal and open Gulf waters are consistent with less riverine phosphate input, seasonal P limitation during periods of hypoxia development (Sylvan et al. 2006, 2007), and the oligotrophic nature of the open Gulf of Mexico (Biggs 1992). In contrast to DIP, concentrations of DOP were not significantly affected by anthropogenic contributions and showed a positive correlation with DOC concentration ($p < 0.01$; see data in Table 1), indicating that the spatial distribution of DOP is related to dissolved organic matter (DOM) input. The highest DOP concentrations occurred in the Pearl River (0.16–0.31 $\mu\text{mol L}^{-1}$), a blackwater stream with higher terrestrially derived DOM inputs (Duan et al. 2007), and in the Bay of St. Louis (0.35 $\mu\text{mol L}^{-1}$). The lowest DOP concentration ($\sim 0.067 \mu\text{mol L}^{-1}$) was found in the Gulf of Mexico, where most of the DOM is supported by autochthonous production.

Concentrations and abundances of COP—Based on the approach for COC (Guo and Santschi 1996), the concentration and abundance of COP can be quantified with the ultrafiltration permeation model for the large-volume

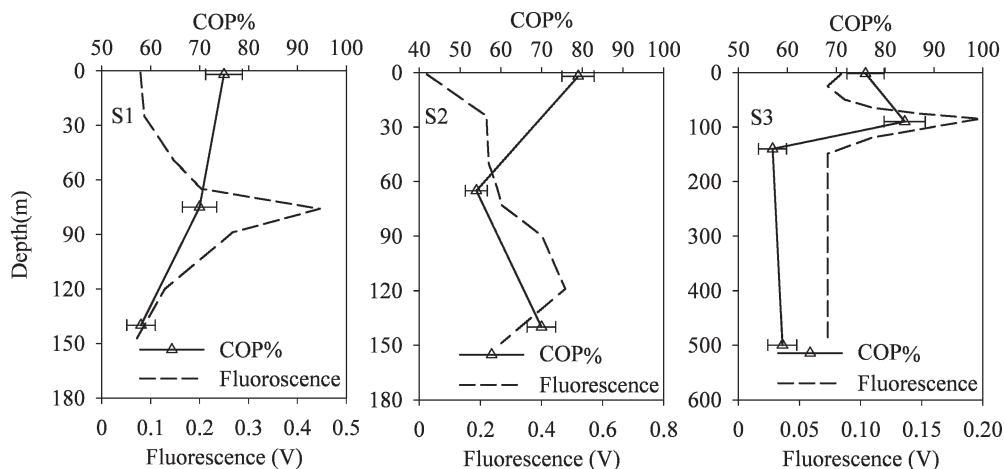


Fig. 2. Variations of colloidal organic phosphorus (COP) fraction and fluorescence intensity (data from CTD sensor) with depth in the Gulf of Mexico at Sta. S1, S2, and S3.

samples (Table 2). Average model-derived COP concentrations (COP_M) were 0.089, 0.21, 0.21, and 0.11 $\mu\text{mol L}^{-1}$ in surface waters of the Mississippi River, the Pearl River, the Bay of St. Louis, and the Mississippi Bight, respectively, corresponding to a respective COP abundance (in terms of %COP in the bulk DOP) of 47%, 88%, 59%, and 67%.

The COP concentrations (COP_C) and abundances (% COP_C) were also calculated with the conventional method (by concentration difference) for all samples to compare with the model-derived COP_M and % COP_M (Table 2). Calculated COP_C concentrations ranged from 0.068 to 0.30 $\mu\text{mol L}^{-1}$, corresponding to a % COP_C that ranged from 52% to 97%.

The COP concentrations and abundances in the open Gulf of Mexico (Sta. 1, 2, and 3) were also quantified with the conventional method because the time-series samples were not available. The COP concentrations in the open Gulf of Mexico were in the range of 0.031–0.061 $\mu\text{mol L}^{-1}$, and abundances were in the range of 54–84% (Fig. 2).

Concentrations and abundances of CIP—Similar to DOP, DIP also follows constant permeation behavior during ultrafiltration (Fig. 3), allowing the quantification of CIP concentration (CIP_M) and abundance (%CIP) with the ultrafiltration permeation model. The CIP concentrations decreased from the rivers (0.067–0.33 $\mu\text{mol L}^{-1}$) to the estuary (0.037 $\mu\text{mol L}^{-1}$) and the coastal waters (0.0006 $\mu\text{mol L}^{-1}$). This result is consistent with the variation trend reported by Suzumura et al. (1998) for CIP in Tokyo Bay.

For samples from the Mississippi River, the Bay of St. Louis, and the Mississippi Bight, the abundance of CIP_M was very low, with a CIP:DIP ratio of 0.01–0.03 (Table 2). Surprisingly, the concentration of CIP_M in the Pearl River was up to 0.33 $\mu\text{mol L}^{-1}$, which was considerably higher than that measured for other sampling sites (Table 2). While there were negligible fractions ($\leq 3\%$) of CIP in both freshwater and seawater samples, the CIP abundance in Pearl River waters was substantial, ranging from 27% to 47% of the total DIP pool (Table 2).

Discussion

Partitioning of dissolved P between inorganic and organic pools—There are two contrasting features in the partitioning of dissolved P between inorganic and organic

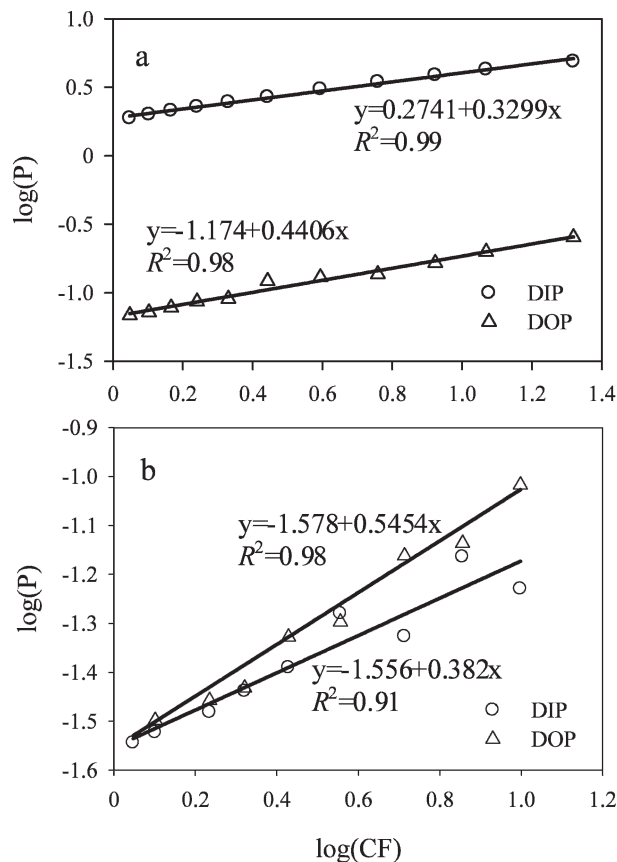


Fig. 3. Examples of permeation behavior of dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) during ultrafiltration. (a) Freshwater sample from the Mississippi River (Mississippi River-2); (b) seawater sample from the Mississippi Bight.

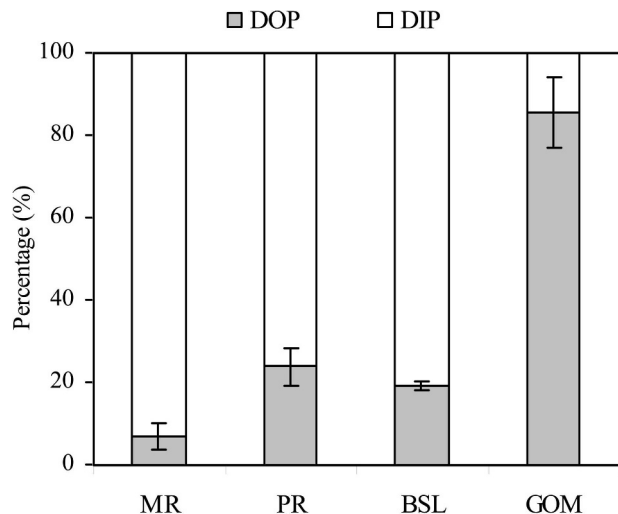


Fig. 4. Partitioning of dissolved phosphorus between inorganic and organic phases in surface waters of the Mississippi River (MR), including the Mississippi and Atchafalaya Rivers, Pearl River (PR), Bay of St. Louis (BSL), and Gulf of Mexico (GOM), including the Mississippi Bight, Mississippi River Plume, and open Gulf of Mexico.

phases. In waters from the Mississippi River and the Bay of St. Louis, DIP made up more than 80% of the TDP pool, indicating a significant contribution of DIP from anthropogenic sources from upstream watersheds. However, the percentage of DIP in the TDP pool dropped to 5–20% in coastal surface waters, and DOP became the dominant P species, comprising up to 80–95% of the TDP pool in surface waters of the Mississippi Bight, the Mississippi River plume, and the open Gulf of Mexico (Fig. 4). The higher DOP fractions in coastal and oceanic environments reflect a combined effect of higher biological production in surface waters, limited DIP inputs, and utilization of DIP by phytoplankton communities.

Quantification of COP—Recent studies have shown that the permeation behavior of LMW solutes, including major ions (Guo et al. 2001), trace metals (Wen et al. 1996; Guo et al. 2000), DOC (Guo and Santschi 1996; Cai et al. 2008a), colored DOM (Belzile and Guo 2006), and standard macromolecules (Guo et al. 2000; Wilding et al. 2004), during ultrafiltration could well be described with the ultrafiltration permeation model. Indeed, based on the DOP and DIP time-series ultrafiltration data set, we have observed that the permeation behavior of both DOP and DIP follows the permeation model (constant permeation). For example, the concentration of LMW DOP in the permeate solution increased with increasing *CF* regardless of the initial DOP concentration (Fig. 3). Bauer et al. (1996) also observed an increase in permeate DOP concentration at higher *CF* (usually 10–20), but attributed it to contamination and scavenging of P into the colloidal fraction during ultrafiltration. Our results further indicate the retention of LMW DOP during ultrafiltration, as is the case for natural DOC and model macromolecules (Guo and Santschi 1996; Guo et al. 2000).

Compared to the COP data obtained from the ultrafiltration permeation model, the conventional method based on discrete sampling and concentration differences could overestimate the %COP for up to 27% (3–27%) depending on the *CF* used during ultrafiltration or could underestimate the %COP (–6%) when substantial COP was lost and the mass balance was much less than 100%. On average, the conventional method overestimated COP abundance of 8% \pm 9% even under the high *CF*s (17–32) used in this study (Table 2). If lower *CF*s were applied, following many previous ultrafiltration studies, the discrepancy between these two estimations would increase exponentially (Belzile and Guo 2006).

Clearly, the reason for COP overestimation is the retention of LMW DOP by the ultrafiltration membrane in the retentate solution, especially under low *CF*s (Guo and Santschi 1996; Belzile and Guo 2006), which is not accounted for by the conventional calculation method. This retention behavior of LMW materials has also been observed for DIP, as shown in Fig. 3, and for major ions, even in the case of few or no colloids (Guo et al. 2001). In contrast, colloidal abundance derived from the permeation model is independent of *CF*. Another caveat with the conventional method is that the calculation relies heavily on the recovery and mass balance, which most studies did not regularly monitor. Our measurements also show that when poor DOP recovery occurred, the %COP_C can be underestimated rather than overestimated (Table 2). Therefore, true colloidal abundance can only be acquired from the application of the ultrafiltration permeation model with time-series permeate sampling, which avoids the errors introduced from LMW species retained under different *CF*s and the effect of poor mass balance.

As shown in Table 3, previous studies using the conventional approach reported a wide range of COP abundance for either the same water sample processed with different ultrafiltration systems or samples at different depths with the same ultrafiltration system. Consequently, the conventional COP estimation approach used in previous studies may hinder the acquisition of accurate COP data and make comparison between COP results impossible. In contrast, our measured COP abundance varied consistently with environmental settings (with an average of 66% \pm 19%), although the sampling sites spanned freshwater from different river basins, estuarine and coastal waters, and open Gulf seawater. For example, the COP abundance varied from 41% to 50% in Mississippi River water samples and increased to 54–84% in the Mississippi River plume and the open Gulf of Mexico (Table 3).

High abundance of CIP in the Pearl River—The high CIP abundance in the Pearl River was unexpected, since orthophosphate, a target molecule, has a molecular weight of much less than 1 kDa and should be partitioned mostly in the <1-kDa LMW phase. The significant amount of CIP observed in the Pearl River likely resulted from the presence of P-containing soil and mineral colloids (Turner et al. 2004). Indeed, previous studies have shown a large amount of colloidal orthophosphate in soil surface runoff and soil extract solutions (Sinaj et al. 1998; Shand et al. 2000; Turner

Table 3. A compilation of colloidal organic phosphorus (COP) in river water and seawater.

Location	Membrane cut-off (kDa)	COP fraction (%)	Reference
Fukuyama Port, Japan	0.5	~55	Matsuda et al. 1985
North Atlantic Ocean	10	20–50	Ridal and Moore 1990
North Pacific Ocean	10	15–100	Ridal and Moore 1992
Woods Hole Coast	1	20–80	Bauer et al. 1996
Tokyo Bay	10	14–36	Suzumura et al. 1998
Pacific Ocean	1	25–50	Clark et al. 1998
Mississippi River and its plume	1	<5	Rinker and Powell 2006
Mississippi River	1	41–50	This study
Pearl River	1	88	This study
Bay of St. Louis	1	59	This study
Mississippi Bight	1	67	This study
Mississippi River plume	1	68–81	This study
Gulf of Mexico	1	54–84	This study

et al. 2004). Thus, a high CIP fraction in the Pearl River is consistent with the fact that the Pearl River is a forested river with fewer anthropogenic influences and fast flushing time from soil to river and has a higher concentration of colloidal iron ($>10 \mu\text{mol L}^{-1}$) than the Mississippi River (about $0.1 \mu\text{mol L}^{-1}$) and other estuarine and coastal waters (subnanomolar to nanomolar level; Wen et al. 1996; Shiller 2003). The extremely high colloidal iron in the Pearl River, also in the form of iron oxyhydroxides (Stolpe et al. unpubl.), could provide sufficient surfaces for DIP sorption and thus induce higher apparent CIP abundance.

Variation and distribution of COP abundance—The Pearl River had the highest COP abundance (up to 88% of DOP), followed by the surface waters in the Mississippi Bight (~67%) and the Bay of St. Louis (59%). The Mississippi River had the lowest COP abundance, ranging from 41% to 50% (Fig. 5). The COP abundance in surface-water samples from the Mississippi River plume and Gulf of Mexico measured $76\% \pm 5\%$, which was quantified with the

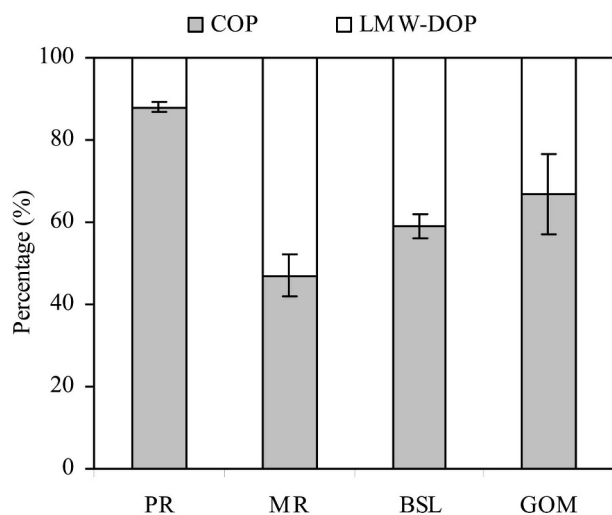


Fig. 5. Spatial variation of COP abundance in the Pearl River (PR), Mississippi River (MR), including the Mississippi and Atchafalaya Rivers, Bay of St. Louis (BSL), and Gulf of Mexico (GOM), including the Mississippi Bight, Mississippi River Plume, and open Gulf of Mexico.

conventional method using higher *CFs* because the time-series samples were not available. These values under high *CFs* are comparable to that measured for the Mississippi Bight (67%). The spatial variation in COP abundance among samples reflected a direct relation to environmental settings.

Comparative studies conducted by Duan et al. (2007) showed that the Pearl River had more fresh DOM input from local terrestrial ecosystems, while the DOM in the lower Mississippi River reflected the integrated inputs throughout the large basin, especially the upstream tributaries, and was subject to more extensive degradation and reprocessing as a result of widespread flood-control structures and intensive agriculture activities. Thus, the low COP abundance in the Mississippi River could result from accumulated photochemical and microbial degradation along with long river transportation. The Mississippi Bight was likely dominated by the autochthonous DOM source, with similar DOC concentration to the Gulf of Mexico samples and a higher fraction of freshly produced polysaccharides in the dissolved carbohydrates (Wang 2009). This autochthonous DOM source may be responsible for the higher COP fraction in the Mississippi Bight (67%). Interestingly, the Bay of St. Louis sample had a COP fraction that was lower than those measured for the Pearl River and the Mississippi Bight, even though its major DOM sources were from blackwater river and autochthonous inputs. Lower COP in the Bay of St. Louis likely resulted from the removal of colloidal materials during estuarine mixing and/or the influence of sewage waters. More studies are needed to better understand the abundance of COP and its biogeochemical cycling in estuarine environments.

Vertical distribution of COP in the Gulf of Mexico showed that the %COP generally decreased with increasing water depth (Fig. 2), similar to the variation pattern of DOC and COC (Guo et al. 1995), reflecting the production of COM in the upper water column and rapid decomposition and remineralization of organic P during downward transportation. Elevated COP seems to coincide with chlorophyll *a* (Chl *a*) maximum depths (based on fluorescence signals) (Fig. 2), indicating in situ contribution of phytoplankton to COP. No significant difference was observed in COP abundance between 140 and 500 m at Sta. S3 (Fig. 2), likely because of the lower DOP

Table 4. Organic carbon:phosphorus ratios in dissolved organic matter (R_D) and colloidal organic matter (R_C) and the fractionation factor between colloidal and dissolved phases (F_{CID}).

Location	R_D	R_C	F_{CID}
Mississippi River	1700 ± 610	2196 ± 954	-0.29 ± 0.10
Pearl River	2724 ± 976	2285 ± 931	0.16 ± 0.06
Bay of St. Louis	943	607	0.36
Mississippi Bight	588	316	0.46
Mississippi River Plume	2346 ± 123	1357 ± 8	0.42 ± 0.02
Gulf of Mexico	1171 ± 129	642 ± 91	0.45 ± 0.07
Pacific Ocean (Sta. Aloha)*	2600	211	0.92

* Data for the Sta. Aloha in the Pacific Ocean are from Sannigrahi et al. (2006).

concentration. Regardless, COP was a dominant DOP fraction in the upper water column, especially in the surface and Chl *a* maximum depths. Colloidal or high-molecular-weight organic matter has been shown to have higher biological and chemical reactivities (Santschi et al. 1995; Amon and Benner 1996). Thus, high COP abundance has important implications in nutrient biogeochemical cycling and the occurrence of hypoxia in the northern Gulf of Mexico, especially under the seasonal P limitation.

C:P ratio of COM—The C:P ratios of COM and bulk DOM ranged from 316 to 2724, which deviated dramatically from the Redfield ratio (106), with a decreasing trend from the Pearl River to the Mississippi River and the Gulf of Mexico (Table 4). In addition, the C:P ratios of COM were consistently lower than those of bulk DOM, except in the case of the Mississippi River samples. Lower colloidal C:P ratios indicate a diagenetically fresher COM component in seawater, while higher C:P ratios in the bulk DOM pool are related to the presence of P-depleted LMW DOM (Sannigrahi et al. 2006).

The C:N:P ratio can be used as an index of the diagenetic status of organic matter and degradation and remineralization pathways of P relative to C and N in natural waters (Clark et al. 1998; Kolowitz et al. 2001; Sannigrahi et al. 2006). As shown in Table 4, terrestrially derived DOM and COM are more depleted in P compared to autochthonous DOM and COM. The lower C:P ratios of both DOM and COM in the Mississippi River compared to ratios for the Pearl River indicate differences in organic sources between these two rivers, with more autochthonous DOM and COM in the Mississippi River and more terrestrial DOM and COM in the Pearl River. These results are supported by the fact that the Pearl River is a forested, blackwater river with higher terrestrial DOM input, while the Mississippi River has a long water residence time and high phytoplankton production (Duan et al. 2007), the latter being a likely result of the effect of flood-control structures and other human influence.

The fractionation factor (F_{CID}) of organic C:P ratio between COM and DOM can be defined as

$$F_{CID} = (R_D - R_C)/R_D \quad (5)$$

where R_C and R_D are the C:P ratios of COM and DOM, respectively. Using available C:P ratio data of oceanic DOM and COM from Sta. Aloha in the Pacific Ocean, a lower C:P ratio in COM compared to DOM gives rise to a F_{CID} value of 0.92 (Table 4). The Pearl River and the seawater samples from the Bay of St. Louis, the Mississippi Bight, the Mississippi River plume, and the Gulf of Mexico had consistently lower C:P ratios in the COM phase compared to those of DOM, with an average F_{CID} value of 0.16 for the Pearl River samples and 0.44 for seawater samples (Table 4). Together with data from the Sta. Aloha (Sannigrahi et al. 2006), differences in organic C:P ratios indicate that the fractionation of molecular composition between COM and DOM is distinct between terrestrial and open-ocean ecosystems, resulting in an increase in the F_{CID} from river waters to estuarine and coastal seawater, and to oceanic waters (Table 4). Since COP is a subfraction of bulk DOP, lower C:P ratios in the COM pool point to a diagenetically younger COM and a degradation pathway of organic matter largely from colloidal or high-molecular-weight to LMW phases. Our conclusion, here derived from organic C:P ratios, is consistent with those reported previously based on evidence of radiocarbon (Santschi et al. 1995) and biological reactivity (Amon and Benner 1996).

In contrast to all other sampling sites, the Mississippi River had a higher C:P ratio in COM but a lower C:P ratio in the bulk DOM pool, resulting in a negative value of F_{CID} (Table 4). The deviation of the Mississippi River F_{CID} value from those of neighboring aquatic environments is somewhat unexpected. Given the dominant terrestrial DOM and COM sources in the river, this deviation may reflect the effect of high DIP levels (approximately four times higher than in the Pearl River), low DOC and COP abundance (Table 1), and river reprocessing of DOM and COM in the Mississippi River. Together with the high DIP:DOP ratio, the negative F_{CID} value and the higher LMW DOP fraction with a lower C:P ratio observed in Mississippi River waters indicates a highly bioavailable DOP pool that may be related to the formation of hypoxia in the northern Gulf of Mexico, especially when primary production is P-limited (Sylvan et al. 2006, 2007).

To the best of our knowledge, our colloidal data set reported represents the first COP and CIP data derived from the permeation model. Our findings of a high percentage of COP and a low C:P ratio in the colloidal phase have important implications for studies of nutrient cycling, hypoxia formation, and environmental effects in the northern Gulf of Mexico. Thus, knowledge of the chemical and phase speciation of P should provide a better understanding of the biogeochemical cycling of P and nutrient limitation and hypoxia formation in coastal ecosystems.

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